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(54) Title: FT-RAMAN SPECTROSCOPIC MEASUREMENT OF OMEPRAZOLE ISOMER RATIO IN A COMPOSITION

(57) Abstract: Fourier Transform Raman Spectroscopy (FT-Raman) determines the isomer ratio of chemical compositions.

FT-RAMAN SPECTOSCOPIC MEASUREMENT OF OMEPRAZOLE ISOMER RATIO IN A COMPOSITION

Cross-Reference to Related Applications

The instant application claims priority to United States Provisional Application Serial No. 60/150,878, filed August 26, 1999, the disclosure of which is incorporated herein by reference in its entirety.

Field of the Invention

The present invention relates to mathematically determining isomeric proportions within a chemical composition using a Fourier Transform Raman Spectrometer (FT-Raman) to create a standard curve.

Background of the Invention

Fourier Transform Raman Spectroscopy (FT-Raman) characterizes chemical compounds by using laser light excitation, which produces elastically scattered light (Rayleigh) and inelastically scattered light (Raman). Filtering within the FT-Raman eliminates intense Rayleigh scattering, which is typically in the range of 10^8 times stronger than the Raman scattering. After passing the filter, a grating disperses the light onto a detector to generate a spectrum. This spectrum provides molecular bonding

information about the sample. Light loses energy to molecular vibration, inducing Raman shift, of $\nu_{laser} - \nu_{scattered} = \Delta\nu_{Raman}$. Frequency range of the FT-Raman ranges from about 4000 cm^{-1} to about 50 cm^{-1} corrected for the Raman laser frequency. Raman use has been disclosed in such patents as United States patent 2,527,121 to Dudenbostel, Jr., the disclosure of which is herein incorporated by reference.

Various compounds used in inhibiting gastric acid secretion are known in the art and include a class of benzimidazole-substituted compounds, one of which is omeprazole. Omeprazole is currently used as the active pharmaceutical ingredient (API) in the commercial United States formulation Prilosec® (manufactured by Merck and Company of Raway, New Jersey). In particular, United States Patent No. 4,255,431 (herein referred to as the '431 patent) proposes such benzimidazole-substituted compounds generally described by the formula (III) in the '431 patent that allegedly encompasses omeprazole. Various methods of making these compounds are proposed in the '431 patent. The disclosure of United States Patent No. 4,255,431 is herein incorporated by reference for the purpose of preparing omeprazole, and pharmaceutical formulations thereof.

Problematic with the '431 patent is that the omeprazole compound has been understood to contain a singular 5-OCH_3 structure on the benzimidazole moiety (*see* Tables 1 and 2, at Ex. 23 of the '431 patent). Standard references also identify omeprazole as exclusively containing this "5-methoxy" structure, including "The United States Pharmacopeia, The National Formulary", USP 24, NF 19 (January 1, 2000) at page 1217; Physicians' Desk Reference®, 51 Edition 1997 at page 516; and "The Merck Index", Twelfth Edition 1996 at page 1174 at entry 6977, the disclosures of these references are herein incorporated by reference. Correct determination of the structure of

the omeprazole (API or drug product) is necessary for proper pharmaceutical use. A recognition of the omeprazole compound as having various or differing isomeric forms in the solid state has heretofore been unrealized, as well as any determination of the amounts of individual isomeric mixtures.

Summary of the Invention

The present invention relates to mathematically determining isomeric proportions within a chemical composition, specifically for an omeprazole composition having a fixed ratio of the 5-methoxy and 6-methoxy isomeric chemical components. The 5-methoxy and 6-methoxy ratio is determined with FT-Raman Spectroscopy to measure, monitor and/or control proper isomeric ratio(s) within omeprazole.

Description of the Drawings

FIG. 1 shows a FT-Raman spectrum between 1330 cm^{-1} and 1390 cm^{-1} for omeprazole compositions of pure 6-methoxy, essentially pure 6-methoxy, 93% 6-methoxy, 88% 6-methoxy (2 spectra), 84% 6-methoxy, and 59% 6-methoxy for the present invention;

FIG. 2 depicts a regression analysis of the average deconvolution of each standard and calculated partial least squares analysis of standard spectra; and,

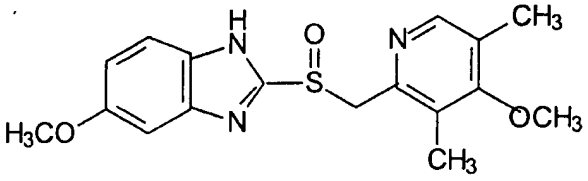
FIGs. **3A** through **3D** show the FT-Raman graph for pure 6-methoxy, 88% 6-methoxy, mannitol and Prilosec®, respectively.

Detailed Description of the Preferred Embodiments

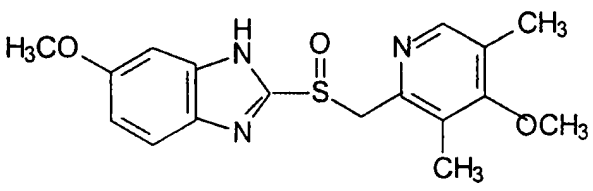
The present invention relates to mathematically determining isomeric proportions

within a chemical composition, specifically for an omeprazole composition having a fixed ratio of the 5-methoxy and 6-methoxy (collectively referred to herein as "5/6-methoxy") isomeric chemical components. The relative amounts of the 5-methoxy and 6-methoxy isomeric components in omeprazole are determined through measurement of, either or both, 5/6-methoxy isomeric levels of an omeprazole composition relative to a standard curve. A Fourier Transform Raman Spectrometer (FT-Raman) is used to characterize the chemical structure of the omeprazole sample, which shows prominent 5/6-methoxy peaks in the range of from about 1345 cm^{-1} to about 1360 cm^{-1} for the 6-methoxy and from about 1360 cm^{-1} to about 1370 cm^{-1} for the 5-methoxy.

Previously, omeprazole was understood to contain only 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole) (referred to herein as "5-methoxy") without containing any 6-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole (referred herein as "6-methoxy"), with the structures shown below:



5-Methoxy



6-Methoxy

It has been unexpectantly discovered that omeprazole comprises an isomeric mixture of 5-methoxy and 6-methoxy isomers of 7:93 ±2%. Heretofore, variations of this ratio have not been known, up to and including a pure 6-methoxy composition of omeprazole. Ratios of the 5/6-methoxy are fixed with the method described herein for a given sample. Isomeric mixtures of omeprazole range from about 0% to about 100% 5-methoxy and from about 0% to about 100% 6-methoxy, such that the sum of the two isomers equals 100%. Other preferred ranges are identified herein. Fixing the ratio of the 5/6-methoxy isomers within omeprazole, API or drug product, allows determination and/or formulation of the proper ratio of the 5/6-methoxy isomers for use in mammals, either for human or animal use.

The 5-methoxy isomer of omeprazole is significantly less stable than the 6-methoxy isomer, and accordingly, degradation of the 6-methoxy isomer generally occurs slower than the 5-methoxy isomer. Degradation products of the 5-methoxy and, to a much lesser extent the 6-methoxy, isomer creates an adverse environment for the stability of the remaining omeprazole (either 5-methoxy or 6-methoxy). This adverse environment created by the degradation products precipitously degrades the remaining omeprazole once the amount of degradation products reaches a certain level, such as

from about 5% or more. As such, proper control of the degradation of omeprazole becomes dependant on fixing the amount of the 5/6-methoxy isomeric ratio within the omeprazole. Accordingly, the amount of 6-methoxy must be fixed within an omeprazole sample to provide reliable stability characteristics.

The ratio of the 5/6-methoxy isomers of omeprazole is quantified within the present invention using a Raman spectroscopic method that was developed using an FT-Raman spectrometer (Nicolet Nexus 670 with a Raman accessory, 1064nm laser, and step and repeat sampling device; Nicolet Instruments Corp. of Madison, Wisconsin). Standards are prepared to establish a standard curve using the FT-Raman. The standard curve is used to evaluate unknown samples of omeprazole. A plurality of standards is required for creating the standard curve, and generally the error of the standard curve is decreased with the greater number of standards used for standard curve calculation. The error within a standard curve may be appreciated by those skilled in the art from the number of standards used, the deviation and/or variation between standards and within a given standard, the 5/6-methoxy ratio difference between standards, the rated resolution of the FT-Raman spectrometer used, and other factors reasonably expected to vary interpretative data in light of the disclosure herein. Generally, a minimum of 4 standards are prepared and used to ensure reliability, with from about 5 or more standard used to more reliably reduce error.

The FT-Raman is used to create the standard curve of the omeprazole composition. Multiple scans and/or replicates may be used and averaged to improve accuracy, such as from about 15 scans or more, more preferably from about 200 scans to about 800 scans, and most preferably from about 400 scans to about 600 scans, or from about 5 replicates or more, more preferably from about 10 replicates to about 50

replicates, and most preferably from about 15 replicates to about 30 replicates, with the determination for the proper number of scans and/or replicates determinable by those skilled in the art. Applicants have surprisingly found that omeprazole varies in amounts, *i.e.*, ratio, of the 6-methoxy and 5-methoxy isomers of the omeprazole composition. Omeprazole is commonly used as an active pharmaceutical ingredient, for use within a drug product. However prior to the present invention, proper determination and quantification of the 5-methoxy and 6-methoxy isomeric components of omeprazole have been unknown. Theoretically omeprazole may range from zero percent (0%) to one hundred percent (100%) 6-methoxy, with the corresponding percentage of 5-methoxy of from 100% to 0%.

As seen in FIG. 1, with the creation of the FT-Raman curve, the isomeric peaks of the 5-methoxy and 6-methoxy isomers of the omeprazole composition are identified. These peaks exist at approximately 1354 cm^{-1} for the 6-methoxy isomeric unit and approximately 1365 cm^{-1} for the 5-methoxy isomeric unit. Measurements are taken of the area under either or both of the two isomeric curves, *i.e.*, 5-methoxy and/or 6-methoxy isomers. Overlap occurs between the 5-methoxy and 6-methoxy isomeric curves which interferes with the direct accurate measurement for the determination of the amounts of the 5-methoxy and 6-methoxy isomers within the omeprazole sample. Accordingly, peak deconvolution algorithms are used to resolve the overlap and permit more accurate measurement.

Additionally, the 5-methoxy and 6-methoxy isomeric peaks of the omeprazole composition were measured relative to "signature peaks" of predominantly non-isomeric components of the omeprazole composition. Peaks from predominantly non-isomeric components of the omeprazole composition were used to provide the relative degree of

emissivity or relative intensity between the 5-methoxy and 6-methoxy peaks. Measurements found that the 5-methoxy and 6-methoxy correlated to one another at approximately 1:1. The predominantly non-isomeric components included measurements of one or more curves such as the peaks at 1587 cm^{-1}, 1627 cm^{-1}, 1185 cm^{-1}, and other identifiable peaks from predominantly non-isomeric components of the omeprazole, as determinable by those skilled in the art particularly in light of noise, excipient interference and/or other chemical additive interference for a particular FT-Raman device and/or omeprazole composition over a given region of the spectrum. Preferably, the peak at approximately 1587 cm^{-1} is used. Multiple peaks may be measured and averaged together.

After creation of the standard curve, unknown isomeric omeprazole compositions were measured and compared to provide the ratio of isomeric components of the 5/6-methoxy in the sample.

Preparation of Standards

Seven standards were prepared using the methods set forth in Standards 1-6 below, plus a commercially available sample of omeprazole purchased from the United States Pharmacopia (USP). Preparation of standards was done to maximize the variance of the 5/6-methoxy ratio between standards. In addition to the USP standard (Standard no. 3, approximately 7% 5-methoxy), a low 5-methoxy isomeric concentration (Standard no. 2, approximately 4-5% 5-methoxy), an extremely low 5-methoxy isomer concentration (preferably in pure form, *i.e.*, 100% 6-methoxy) (Standard no. 1, approximately 0% 5-methoxy), at least one high concentration 5-methoxy isomer concentration (Standard no. 7, approximately 40-50% 5-methoxy), and two or more standards distributed over the range from about 5% to about 30% 5-methoxy (Standard

nos. 4-6, approximately 12, 16 and 16.5% 5-methoxy, respectively). To establish the standard curve, each standard was run with at least triplicate preparations with at least 15 replicates for each standard preparation and at least 500 scans per replicate, using a resolution of 2 cm^{-1}, using the step and repeat sampling device in the continuous mode with instrument parameters set to generate an acceptable signal to noise (S/N).

Standard 1: Preparation of Pure 6-methoxy

To a 1000mL glass bottle having a screw cap having about 300mL of methanol was added 1.93g of sodium hydroxide pellets. The solution was stirred until such pellets dissolved, and omeprazole API was added until a heavy suspension was formed. The solution was capped and allowed to sit, at ambient temperature, for four days, then filtered using vacuum filtration and a paper filter. The resulting solid was washed with three, 50mL portions of methanol, then placed in a vacuum oven to dry at ambient temperature. The compound was removed after drying for 24 hours, and the purity confirmed by FT-Raman spectroscopy. All samples are shown to be pure 6-methoxy by Raman spectroscopy.

Standard 2: Preparation of Essentially Pure 6-methoxy (4%-6% 5-methoxy)

Approximately 850mL of methanol was placed in a 1 liter glass bottle with a screw cap. The solution was saturated by dissolving approximately 10.5g of 5/6-methoxy, and the resulting solution was stirred. Once the solution was saturated, an additional 17g of 5/6-methoxy was added to the saturated solution to create a suspension. The cap was sealed and the saturated suspension was allowed to stir and equilibrate for about four days.

After four days, the suspension was filtered through a paper filter and then washed with a small amount of methanol. The supernatant was returned to the 1 liter

glass bottle and an additional 10g of 5/6-methoxy was added to the saturated solution. The procedure was repeated to create additional sample. All samples are shown to be essentially pure 6-methoxy by Raman spectroscopy. This procedure has also been successfully carried out using ethanol.

Standard 3: United States Pharmacopia (7%-8% 5-methoxy)

Commercially available sample of omeprazole purchased from the United States Pharmacopia (USP).

Standard 4: Preparation of 5/6-methoxy (11%-13% 5-methoxy)

To a 50mL beaker was added about 1g of 5/6-methoxy to 30mL of methylene chloride. Additional 5/6-methoxy was added to the resulting solution until a suspension of the material was formed. The solution was stirred for approximately 10 minutes, and then filtered through a 0.45μm Poly(tetrafluoroethylene) (PTFE) or Nylon filter. The resulting saturated solution was placed in a shallow petri dish, covered and stored under refrigerated conditions (approximately 5°C) and a humidity range of approximately 50 to 90 percent until crystals formed (between 1-2 days). The identity of the compound was confirmed by single crystal x-ray diffraction indicating the resulting material to contain between about 81 and 86 percent (w/w) of the 6-methoxy and between about 14 and 19 percent (w/w) of 5-methoxy. Deconvolution of Raman spectroscopy showed the resulting material to contain approximately 88 percent (w/w) of the 6-methoxy and approximately 12 percent (w/w) of 5-methoxy.

Standard 5: Preparation of 5/6-methoxy (15%-17% 5-methoxy)

To a 50mL beaker was added about 1g of 5/6-methoxy to 30mL of acetone. Additional 5/6-methoxy was added to the resulting solution until a suspension of the material was formed. The solution was stirred for approximately 10 minutes, and then

filtered through a 0.45μm Poly(tetrafluoroethylene) (PTFE) or Nylon filter. The resulting saturated solution was placed in a shallow petri dish, covered and stored under refrigerated conditions (approximately 5°C) and a humidity range of approximately 50 to 90 percent until crystals formed (between 1-2 days). The identity of the compound was confirmed by single crystal x-ray diffraction indicating the resulting material to contain between about 79 and 82 percent (w/w) of the 6-methoxy and between about 18 and 21 percent (w/w) of 5-methoxy. Deconvolution of Raman spectroscopy showed the resulting material to contain approximately 84 percent (w/w) of the 6-methoxy and approximately 16 percent (w/w) of 5-methoxy.

Standard 6: Preparation of 5/6-methoxy (15%-17% 5-methoxy)

To a 50mL beaker was added about 1g of 5/6-methoxy to 30mL of ethanol containing 1mL of ammonium hydroxide. Additional 5/6-methoxy was added to the resulting solution until a suspension of the material was formed. The solution was stirred for approximately 10 minutes, and then filtered through a 0.45μm Poly(tetrafluoroethylene) (PTFE) or Nylon filter. The resulting saturated solution was placed in a shallow petri dish, covered and stored under refrigerated conditions (approximately 5°C) until crystals formed (between 2-6 days). The identity of the compound was confirmed by single crystal x-ray diffraction indicating the resulting material to contain between about 85 and 88 percent (w/w) of the 6-methoxy and between about 12 and 15 percent (w/w) of 5-methoxy. Deconvolution of Raman spectroscopy showed the resulting material to contain approximately 84 percent (w/w of the 6-methoxy and approximately 16 percent (w/w) of 5-methoxy.

Standard 7: Preparation of 5/6-methoxy (40%-50% 5-methoxy)

To a 50mL beaker was added about 1g of 5/6-methoxy to 30mL of chloroform.

Additional 5/6-methoxy was added to the resulting solution until a suspension of the material was formed. The solution was stirred for approximately 10 minutes, and then filtered through a 0.45μm Poly(tetrafluoroethylene) (PTFE) or Nylon filter. The resulting saturated solution was placed in a shallow petri dish, covered and stored under refrigerated conditions (approximately 5°C) and a humidity range of approximately 50 to 90 percent until crystals formed (between 1-2 days). The identity of the compound was confirmed by single crystal x-ray diffraction indicating the resulting material to contain between about 50 and 60 percent (w/w) of the 6-methoxy and between about 40 and 50 percent (w/w) of 5-methoxy. Deconvolution of Raman spectroscopy showed the resulting material to contain approximately 58 percent (w/w) of the 6-methoxy and approximately 42 percent (w/w) of 5-methoxy.

API 5/6-methoxy Determination

Raman spectra were created for each selected standard as specified. Except for the pure 6-methoxy isomer standard, a deconvolution algorithm was used to deconvolute the peak areas of the peaks at approximately 1365 cm^{-1} for the 5-methoxy isomer and approximately 1354 cm^{-1} for the 6-methoxy isomer. The pure 6-methoxy showed a single peak at about 1354 cm^{-1}, and as such the percent 6-methoxy was set at a concentration of 100 percent. A software program capable of analyzing Raman spectra in deconvolution algorithm format such as, for example, Nicolet's TQ Analyst™, was used to generate an area percentage of the 5-methoxy relative to the total area of the 5/6-methoxy isomer of each non-pure 6-methoxy standard for the 5/6-methoxy isomer. The area percent was visually checked against the curve to ensure that the measured amounts rationally compared with the curve. The standard deviation for each set of replicates for a standard was less than about 0.7%, and the average standard deviation for the average of

all runs and replicates of a given standard was less than about 0.7%.

A software program capable of analyzing Raman spectra in a partial least squares format such as, for example, Nicolet's TQ Analyst™, was used to generate a standard curve using the average determined percent 6-methoxy isomer values and the spectrum of a given standard, and to assure accuracy, the correlation coefficients should be at or above about 0.98 among all standards for a given method. FIG. 2 depicts a regression analysis of the average deconvolution and the calculated partial least squares analysis of standard spectrum.

Each omeprazole sample was then analyzed using the method described for establishing the standards, except at least 5 replicates per sample preparation were used, and at least 100 scans per replicate with at least triplicate preparations per sample were used. Using the above-referenced partial least squares analysis, the percent 6-methoxy isomer, and thus the percent 5-methoxy isomer was determined for each scan and the average of the 15 spectra was calculated. The standard deviation (SD) for each scan set of replicates was less than about 1.0%, and the average standard deviation of all runs and replicates of a given sample was less than about 1.0%. High standard deviation values are an indication of variability which may be caused by small amounts of sample burning. When burning is suspected, the preparation should be repeated.

Using the methods taught above, results from establishing the standard curve are as follows:

Standard	% 5-methoxy	% 6-methoxy	Standard Deviation (SD)
Standard 1	0.000	100.000	0
Standard 2	5.875	94.125	0.338
Standard 3 (USP)	7.250	92.750	0.556

Standard 4	12.246	87.754	0.505
Standard 5	16.005	83.995	0.501
Standard 6	16.413	83.587	0.597
Standard 7	41.673	58.327	0.328

Using the API quantitative method taught above, 3 lots of omeprazole API (commercial API lots from Merck and Company, Raway, New Jersey) were analyzed. Results are as follows:

Sample/Lot	% 5-methoxy	% 6-methoxy	Standard Deviation (SD)
01	7.50	92.50	0.77
02	8.02	91.98	0.56
03	7.61	93.39	0.81

Results from these data through the above-described quantitative method confirmed that the compound known as omeprazole is not 5-methoxy as previously designated (*e.g.*, USP standard for omeprazole and 3 lots of omeprazole API from the sole United States manufacturer of omeprazole), but rather a 5/6-methoxy in a tightly defined ratio of about 7:93 ± from about 2% of the 5-methoxy isomer and 6-methoxy isomer, respectively.

As seen in FIG. 1, a shift in frequency the 5-methoxy and 6-methoxy curves also is detectable in proportion to the ratio amount of the two isomeric components, ranging between 1353 cm^{-1} for pure 6-methoxy to 1354 cm^{-1} for 40% 5-methoxy. The frequency of the maxima of individual peaks and minima of the valleys shift to high or lower wavenumbers depending on the relative percentage of 5/6-methoxy in the standards. Correlations of many of the prominent peaks were examined between standards with

consistent results. However, the small variations in the wavenumbers between sample and standards allowed for relatively large errors in calculations. With increased accuracy of the detecting FT-Raman, this frequency shift becomes useful in quantifying the 5/6-methoxy ratio.

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API and Drug Product 5/6-methoxy Determination

An additional method for quantifying the ratio of the 5/6-methoxy isomers of omeprazole API, albeit less precise than the quantitative FT-Raman method previously described herein, as well as ratio of the 5/6-methoxy isomers in omeprazole drug product was developed. This method also used an FT-Raman spectrometer (Nicolet Nexus 670 with a Raman accessory, 1064nm laser, and step and repeat sampling device). This method, too, is conducted in three stages: preparation of standards, establishing a standard curve, and analysis of samples. Typically a minimum of 4 to 5 standards are prepared.

The present FT-Raman method for API and drug product analysis uses the same method set forth above, including preferred aspects, as the method set forth for the more quantitative API method relative to the number of preparations and scans per replicate of each standard, resolution, sampling device, deconvolution of standard peaks, determination of peak area, and standard deviation for each set of replicates and the average of all runs and replicates of a given standard.

Rather than a partial least squares analysis, however, a software program capable of analyzing Raman spectra in a corrected classical least squares format, for example, Nicolet's TQ Analyst™, was used to generate a standard curve using the determined percent 6-methoxy isomer values and the spectrum of a given standard. The method is performed by a ratio of a main omeprazole band (such as, for example, approximately 1627 cm^{-1}) to a second suitable omeprazole band (such as, for example, the peak at about 1587 cm^{-1}). In the event the presence and magnitude of the matrices from pharmaceutical excipients in drug product interfere with the resolution of the peak related to the 6-methoxy isomer and/or the preferred internal omeprazole band, other sets of bands, such

as 1587 cm^{-1} and 1201 cm^{-1}, respectively, and 1185 cm^{-1} and 1512 cm^{-1}, respectively, may be used. Correlation coefficients were at or above about 0.98 among all standards.

For omeprazole API, each sample is prepared under the same instrument conditions as the standards except it is preferred to use at least 5 replicates per sample preparation and at least 100 scans per replicate. Using the above-referenced corrected classical least squares analysis, the percent 6-methoxy isomer, and thus the percent 5-methoxy isomer, was determined for each scan, and the average of the 15 spectra is calculated. The standard deviation for each set of replicates is less than about 2.0%, and the average standard deviation of all runs and replicates of a given sample was less than about 2.0%.

For omeprazole drug product, capsules and tablets are similarly prepared. For capsules, a sufficient number of capsules, preferably about 5-10 capsules, are opened and the omeprazole beads are emptied into an appropriate container. The container is gently rolled to mix the beads or powder, as appropriate, from the various capsules to provide a generally homogeneous blend. For tablets, a sufficient number of tablets, preferably about 5-10 tablets, are gently ground (vigorous grinding may affect the ratio of 5/6-methoxy isomers in omeprazole), and blended to provide a generally homogeneous blend of the ground material.

Each appropriate composite sample was analyzed under the same instrument conditions as the standards, adjusting to an appropriate laser wattage to compensate for the presence of excipients. For FT-Raman analysis, each sample preparation (the composite from capsules or tablets) was run using at least triplicate preparations with at least 3 replicates and at least 500 scans per replicate. Using a corrected classical least squares analysis, the percent 6-methoxy isomer, and thus the percent 5-methoxy isomer,

was determined for each scan, and the average of the 9 spectra was calculated. The standard deviation for each set of replicates was less than about 3.0%, and the average standard deviation of all runs of a given sample was less than about 3.0%.

Although the partial least squares method described above is more accurate than this classical least squares method, the deconvolution of the peaks related to the 5-methoxy and 6-methoxy isomers remains the same for both methods and, thus, the standard curve remains the same. Results from API sample analysis with the classical least squares method showed a slightly lower bias than the results from using the partial least squares method, but the data from the partial least squares analysis of omeprazole API samples confirmed the validity of this method for a generally quantitative method for determining the ratio of 5/6-methoxy isomers in omeprazole drug product (Prilosec®) which is commercially available via prescription. The drug product used in the present classical least squares method was provided by Merck and Company of Raway, New Jersey.

Results from API analysis using the classical least squares methods are as follows:

Sample/Lot	% 5-isomer	% 6-isomer	Standard Deviation (SD)
04	6.14	93.86	0.97
05	6.56	93.44	1.10
06	6.40	93.60	1.21

When applying this classical least squares analytical method to drug product, it was unexpectedly discovered that the ratio of 5/6-methoxy isomers of omeprazole becomes significantly influenced by a multitude of factors during the preparation of drug

product (final pharmaceutical formulations for administration, preferably in unit dosage form).

For the sole omeprazole drug product registered by the U.S. Food and Drug Administration and sold in the United States (Prilosec®), the ratio of the 5/6-methoxy isomers in API typically shifts from a ratio of about 7:93 (± about 2%), for the 5-methoxy and 6-methoxy respectively, to a ratio in drug product of about 14:86 (± from about 3%), for the 5-methoxy and 6-methoxy respectively. Factors such as mechanical manipulation (*e.g.*, grinding or, potentially, aggressive sieving) and, particularly the use of commonly used wet granulation processes during drug product preparation have likely contributed to this significant and unexpected shift.

Various physical conditions may be manipulated during the subjecting step to govern the amount of the 5-methoxy compound, *e.g.*, revolutions per minute (RPM) and length of subjecting step. The subjecting step is preferably carried out from about 350 rpm to about 500 rpm, more preferably from about 350 rpm to about 450 rpm, and most preferably about 450 rpm. A preferred time for carrying out the subjecting step is from about 5 to about 30 minutes, more preferably from about 10 min to about 30 min, and most preferably about 15 minutes. Advantageously, the compounds are not degraded during this operation. The subjecting step may be carried out by various machines that apply appropriate grinding energies to solid materials. Preferably, the machine is a mechanical grinder. One example of a suitable grinder is set forth in U.S. Patent No. 5,773,173 to Whittle et al., the disclosure of which is incorporated herein by reference in its entirety. It should be appreciated that one may employ embodiments other than those described above for forming such compounds of the present invention in solid state.

Shifts from the more thermodynamically stable compounds, with having a higher percentage of the 6-methoxy isomer (with the pure 6-methoxy isomer being preferred), to the less stable compounds having increasing concentrations of the 5-methoxy isomer in the same composition can affect the stability and dissolution profiles of drug product. Compounds and pharmaceutical formulations of the present invention having such higher percentage of such 6-methoxy isomer provide greater stability.

Using the classical least squares analytical method described above, results for Prilosec® drug product are as follows:

Prilosec Dosage	% 5-methoxy	% 6-methoxy	Standard Deviation (SD)
20mg	14.7	85.3	2.3
20mg	14.5	85.5	2.0
20mg	14.7	85.3	3.0
40mg	13.2	86.8	1.6
40mg	12.9	87.1	0.9
10mg	13.6	86.4	2.8
10mg	13.3	86.7	2.4

Additionally, a homogeneous dry blend pharmaceutical formulation of the above-referenced omeprazole API from Merck and Company and mannitol was prepared with an equivalent dose of 20 mg per dosage form. Using FT-Raman, as disclosed herein, the ratio of the 5/6-methoxy was determined using the corrected classical least squares method. It was unexpectedly found that the 5/6-methoxy ratio of the dry blend remained the same as the API (approximately 6-7% 5-methoxy and 93-94% 6-methoxy), because the percentage of 5/6-methoxy between Merck and Company API varies from the formulated Prilosec® drug product.

As such, the 5/6-methoxy ratio may be fixed within appropriate pharmaceutical formulations, including compound(s), composition(s) and/or complex(es) of the omeprazole API, at least one metal cation, preferably an alkaline metal cation, especially sodium or magnesium of pharmaceutically acceptable salts, solvates, hydrates, or combinations thereof, and preferably at least one non-aqueous pharmaceutically acceptable carrier, diluent or excipient. Preferably this includes dry blends of the pharmaceutical formulations, which may or may not have varied amounts of 5/6-methoxy ratios as between the API and drug product. Stabilizing agents well known in the pharmaceutical art may be optionally added and blending of the drug product may be moderated to minimize degradation of the omeprazole. Such blended mixture is then directly compressed into a tablet or prepared into other pharmaceutically acceptable dosage forms or, preferably, encapsulated using standard preparation techniques. The final pharmaceutical dosage form, when to be used for oral administration, is then optionally and preferably enterically coated. Such pharmaceutical formulations of the present invention are preferably prepared into unit dosage forms comprising the active ingredient(s) concentrations taught herein, preferably in the range from about 5mg to about 60mg per unit dose and at least one aquesou or non-aqueous carrier, diluent, or pharmaceutically acceptable excipient. Dry blends used to maintain essentially the same ratio of the 5/6-methoxy ratio from API to dry product preferably use non-aqueous carriers, diluents, or pharmaceutically acceptable excipients, whereas other dosage forms, including preferred oral dosage forms, use either non-aqueous or aqueous carriers, diluents, or pharmaceutically acceptable excipients. More preferred concentrations range from about 8mg to about 10mg, about 16mg to about 20mg, and from about 32mg to

about 40mg per unit dose, while unit doses of about 10mg, 20mg, and 40mg are especially preferred.

Such pharmaceutical formulations, particularly in unit dosage form, are used for treating (including prophylaxis) the disease states described herein. As such, the present invention further provides methods of treating a subject (e.g., mammals, particularly humans) comprising administering to a subject in need of treatment of gastric acid related diseases and/or disease states, a therapeutically effective, non-toxic amount of the aforementioned pharmaceutical formulations. Preferred compounds and compositions, as active ingredients, unit dosage forms, and dosage strengths are determinable by those skilled in the art in light of the disclosure herein.

The following examples show omeprazole formed in different ratios of the 5/6-methoxy as analytically determined. As such the 5/6-methoxy ratio within omeprazole preparations may be controlled allowing omeprazole to be fixed with a specified ratio of 5-methoxy and 6-methoxy isomeric compounds.

The following are tables (1A and 1B) of exemplary ranges for omeprazole, with Table 1A listing possible ranges of 5/6-methoxy in API and Table 1B listing possible 5/6-methoxy in Drug Product:

TABLE 1A (API)

5-methoxy (%)	6-methoxy (%)
100 to 0	0 to 100
90 to 0	10 to 100
5 to 0	95 to 100
100 to 9	0 to 91
50 to 0	50 to 100
50 to 10	50 to 90
45 to 12	55 to 88
40 to 18	60 to 82
4 to 1	96 to 99 (Essentially Pure)
2 to 0	98 to 100 (Pure)

TABLE 1B (Drug Product)

5-methoxy (%)	6-methoxy (%)
100 to 0	0 to 100
90 to 0	10 to 100
10 to 0	90 to 100
100 to 15	0 to 85
100 to 20	0 to 80
50 to 15	50 to 85
50 to 20	50 to 80
40 to 18	60 to 82
4 to 1	96 to 99 (Essentially Pure)
2 to 0	98 to 100 (Pure)

Especially preferred is API that ranges from about 96% or more and/or about 91% or less 6-methoxy, and drug product ranging from about 89% or more and/or about 83% or less 6-methoxy.

Omeprazole compounds of the present invention, as described herein may be used within the pharmaceutical formulations, such as tablets, pills, powders, elixirs, suspensions, emulsions, solutions, syrups, or capsules, of the omeprazole API, with

suitable pharmaceutical formulations determinable by those skilled in the art. Determination of the 5/6-methoxy ratio becomes more difficult with the presence of carriers, diluents, excipients, and/or other compositions used in the omeprazole formulation, such as starches, gum arabic, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, mannitol, sorbitol, sucrose, dextrose, and the like. Dosage forms are known in the art, such as a unit dosage form, each dosage containing from about 5mg to about 60mg, such as from about 8mg to about 10mg, about 16mg to about 20mg, and about 32mg to about 40mg, especially 10mg, 20mg, and 40mg per dosage unit. The term "unit dosage form" refers to physically discrete units, such as capsules or tablets suitable as unitary dosages for human patients and other mammals, with each unit containing a predetermined quantity of one or more active ingredient(s) calculated to produce the desired therapeutic effect, in association with at least one pharmaceutically acceptable carrier, diluent, excipient, or combination thereof. Omeprazole is known for the treating a subject (*e.g.*, mammal, particularly humans) for a number of disorders, particularly for preventing and treating gastric acid related diseases. The amount given to a particular patient may be determined by an attending physician or other qualified person to administer a therapeutically effective amount of the omeprazole.

FIGs. **3A** through **3D** show the FT-Raman graph for pure 6-methoxy, 88% 6-methoxy, mannitol and Prilosec®, respectively over a range of about 800 cm^{-1} to about 1400 cm^{-1}. As seen in FIGs. **3A** through **3D**, selection of areas of the spectra for analysis is determined relative to the excipients in the drug product.

The foregoing summary, description, examples and drawings of the invention are not intended to be limiting, but are only exemplary of the inventive features which are defined in the claims.

What is claimed is:

1. A Fourier Transform Raman Spectroscopy method for mathematically determinating isomers within a chemical composition comprising the steps of:

creating a standard curve;

analyzing a chemical sample, wherein the isomeric ratio is determined.

2. The method of claim 1, wherein the chemical sample comprises omeprazole and the ratio comprises a ratio of the 5-methoxy and 6-methoxy isomers of omeprazole on the benzimidazole moiety.

3. An omeprazole API composition fixed with a ratio of 5-methoxy and 6 methoxy isomers.

4. The omeprazole API composition of claim 3, fixed with a ratio of from about 4 percent or less 5-methoxy and from about 96 percent or more 6-methoxy.

5. The omeprazole API composition of claim 3, fixed with a ratio of from about 9 percent or more 5-methoxy and from about 91 percent or less 6-methoxy.

6. An omeprazole API composition having a predetermined ratio of 5-methoxy and 6-methoxy.

7. A pharmaceutical formulation comprising an omeprazole composition fixed with a ratio of 5-methoxy and 6-methoxy isomers.

8. The pharmaceutical formulation of claim 7, fixed with a ratio of from about 11 percent or less 5-methoxy and from about 89 percent or more 6-methoxy.

9. The pharmaceutical formulation of claim 7, fixed with a ratio of from about 17 percent or more 5-methoxy and from about 83 percent or less 6-methoxy.

10. A pharmaceutical formulation comprising an omeprazole composition having a predetermined ratio of 5-methoxy and 6-methoxy.

11. A method for establising a fixed ratio of the 5-methoxy and 6-methoxy isomers of omeprazole on the benzimidazole moiety of omeprazole comprising the comprising the steps of:

creating a standard curve;

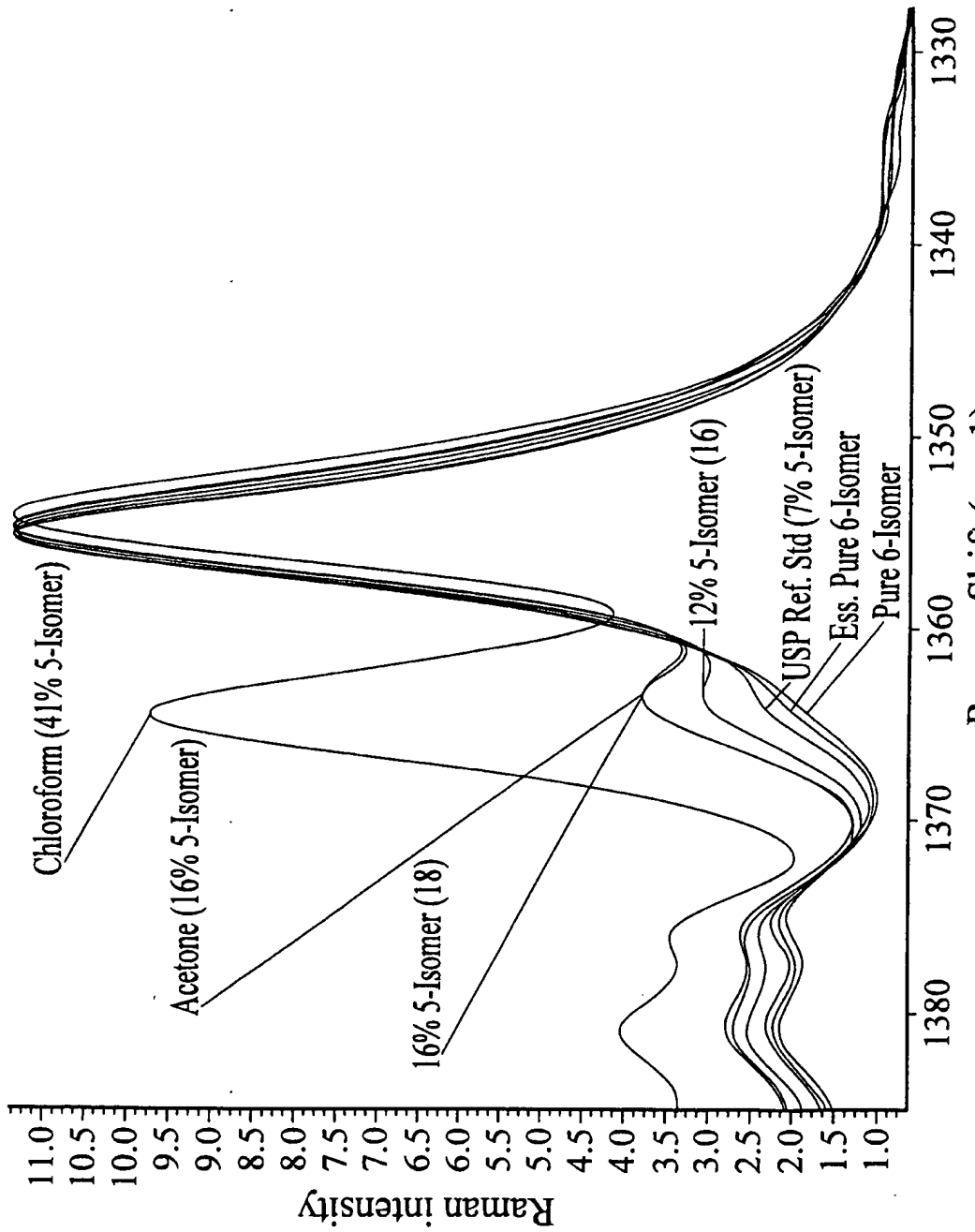
analyzing a chemical sample, wherein the isomeric ratio is determined.

12. The method of claim 11, wherein the omeprazole compound is a pharmaceutical formulation comprising omeprazole and one or more pharmaceutical salts, solvates, hydraes, or combiations thereofand at least one pharmaceutically acceptable carrier, diluent, or excipient.

13. The method of claim 12, wherein a non-toxic, therapeutically effective amount of the

pharmaceutical formulation is administered to a mammal in need of treatment for gastric acid related diseases.

14. The method of claim 13, wherein a non-toxic, therapeutically effective amount of the pharmaceutical formulation is administered to a mamal for the inhibition of gastric acid.


Chloroform (41% 5-Isomer)
Acetone (16% 5-Isomer)
16% 5-Isomer (18)
12% 5-Isomer (16)
USP Ref. Std (7% 5-Isomer)
Ess. Pure 6-Isomer
Pure 6-Isomer
Raman intensity
Raman Shift (cm-1)
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
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2.0
1.5
1.0
1380
1370
1360
1350
1340
1330

FIG. 1

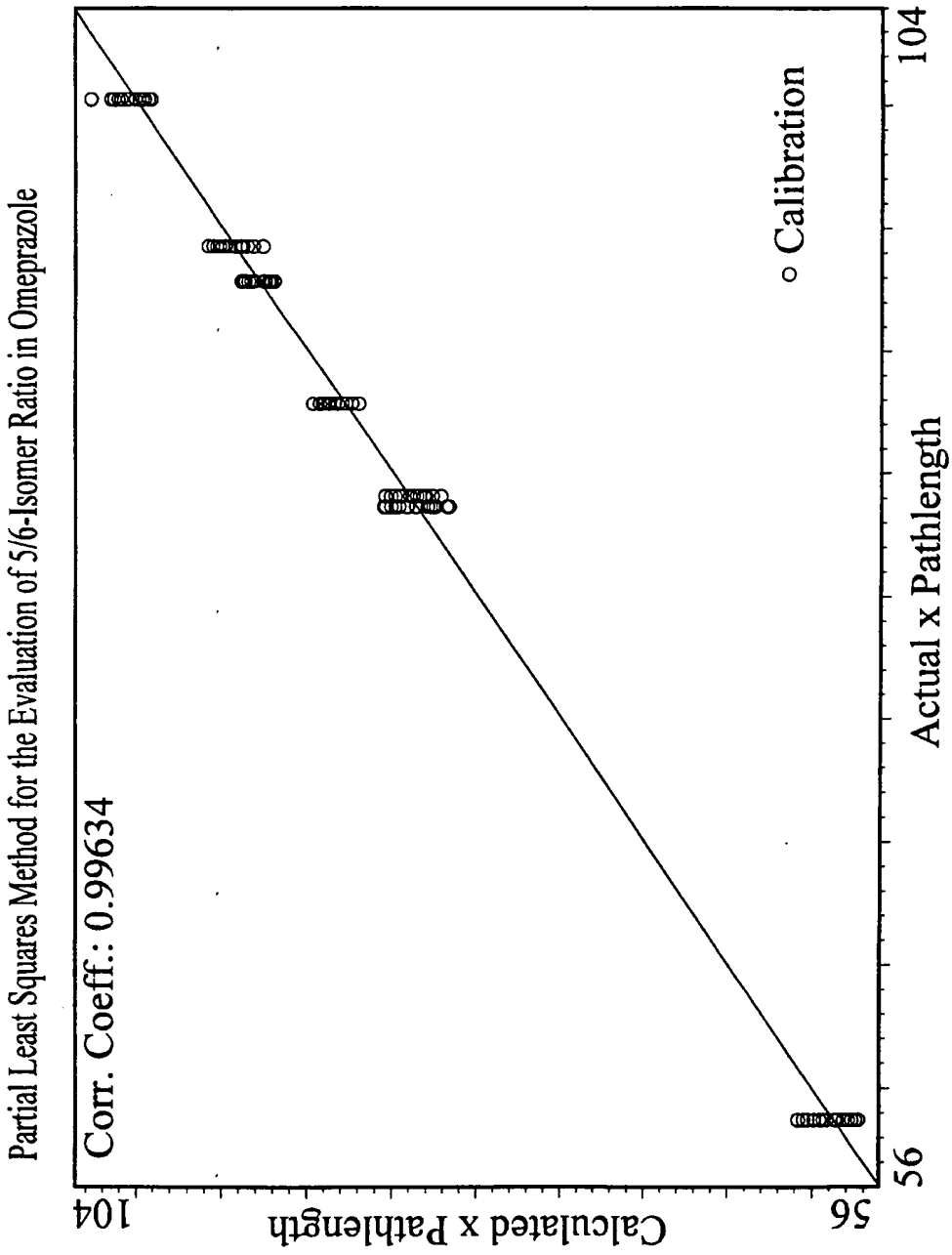
Partial Least Squares Method for the Evaluation of 5/6-Isomer Ratio in Omeprazole
Corr. Coeff.: 0.99634
Calculated x Pathlength
Actual x Pathlength
Calibration
104
56
104
56

FIG. 2

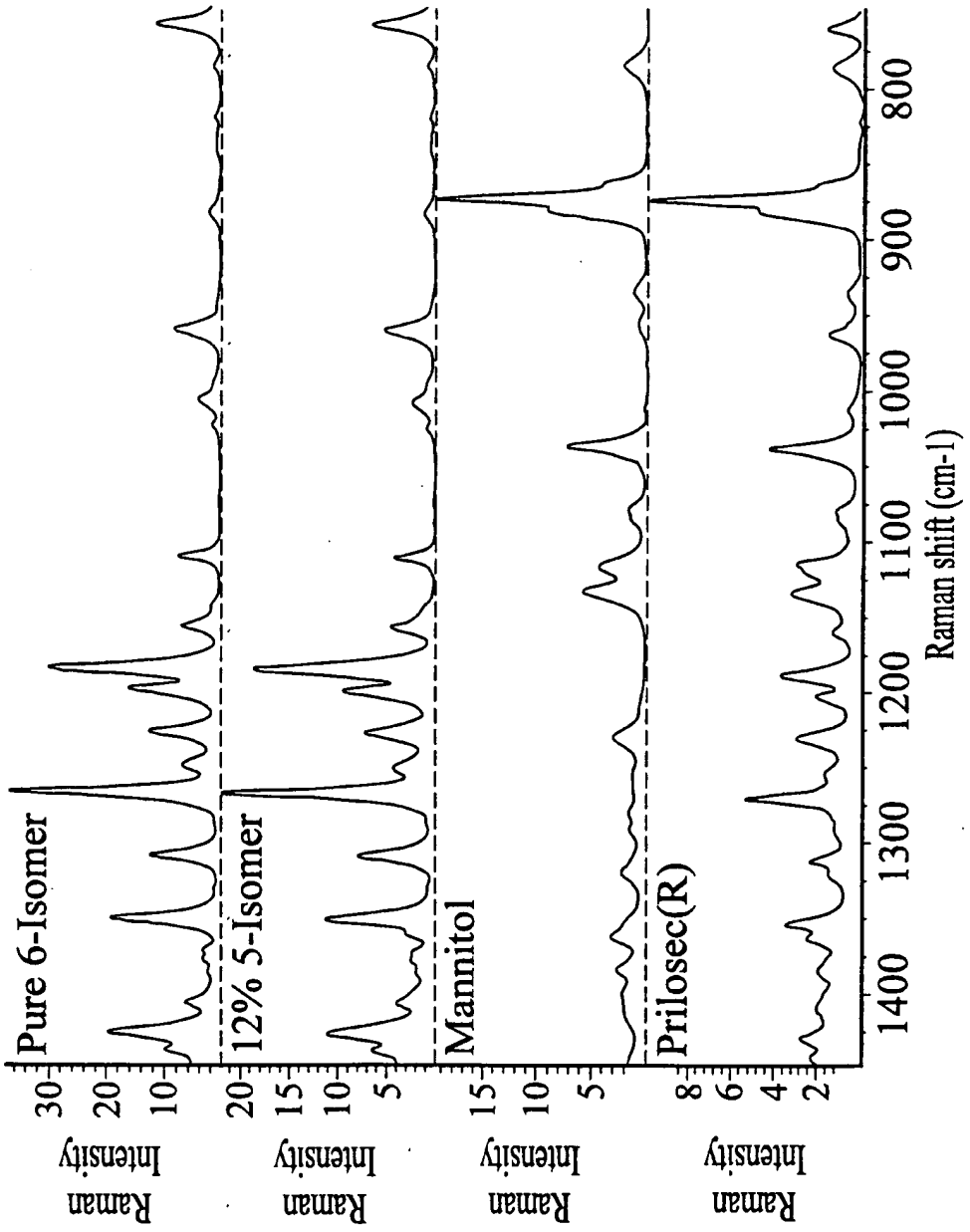
Pure 6-Isomer
12% 5-Isomer
Mannitol
Prilosec(R)
Raman Intensity
Raman shift (cm-1)
1400
1300
1200
1100
1000
900
800

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/23368

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/4439 G01N21/65 G01N33/15

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	US 5 850 623 A (H. SMITH CARMAN ET AL) 15 December 1998 (1998-12-15) column 19 -column 22 claim 21 --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 December 2000	02/01/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Siatou, E

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/23368

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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